

-continued

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We claim:

1. An aptamer that binds both cocaine and 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND), wherein said aptamer is derived from MNS-4.1, wherein said derivation comprises the conversion of at least one non-canonical base pair to a Watson-Crick base pair, wherein the aptamer is not modified at either the T21 or the A23 position relative to MNS-4.1, wherein said aptamer has increased stability, and wherein said aptamer binds cocaine more strongly than ATMND.

2. The aptamer, according to claim 1, wherein the equilibrium dissociation constant for binding to cocaine is 5.0 μ M or less.

3. The aptamer, according to claim 1, wherein at least 95% of the fluorescence of the ATMND is quenched in the absence of cocaine.

4. The aptamer, according to claim 1, which is 38-GC or 38-GT.

5. A method for detecting cocaine in a biological sample wherein said method comprises contacting said sample with an aptamer according to claim 1 to which 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) is bound and determining whether an increase in fluorescence occurs, wherein an increase in fluorescence is indicative of the presence of cocaine in the sample.

6. The method, according to claim 5, wherein said aptamer is derived from MNS-4.1, wherein said derivation comprises the addition of complementary base pairs at multiple sites that confer upon the aptamer increased stability, wherein the aptamer is not modified at either the C21 or the T20 position relative to MNS-4.1.

7. The method, according to claim 5, wherein the aptamer is 38-GC or 38-GT.

8. The method, according to claim 5, wherein a molar ratio of the aptamer to ATMND of 8:1 is utilized.

9. The method, according to claim 5, wherein the biological sample is selected from the group consisting of saliva, urine, and serum.

10. The method, according to claim 5, wherein at least 95% of the fluorescence of the ATMND is quenched when cocaine is not present.

11. The method, according to claim 5, wherein a signal gain of at least 10 is obtained in the presence of 50 μ M of cocaine.

12. A cooperative-binding split aptamer (CBSA) derived from two molecules of MNS-4.1, the CBSA having a short and a long fragment, wherein the aptamer comprises two cocaine binding domains and an ATMND-binding site when the short and long fragment associate in the presence of cocaine; wherein cocaine binding to a first cocaine binding domain greatly increases the affinity of the second cocaine binding domain; wherein the CBSA comprises at least one non-canonical base pair converted to a Watson-Crick base pair; wherein the CBSA is not modified at either of the positions corresponding to T21 and A23 of MNS-4.1; and wherein the CBSA further comprises at least one additional Watson-Crick base pair added between the two cocaine-binding domains.

13. The aptamer, according to claim 12, wherein the addition of one Watson-Crick base pair confers enhanced cocaine-induced aptamer assembly.

14. The cooperative-binding split aptamer according to claim 12, wherein said short fragment comprises a quencher at the 5' terminus and a fluorophore at the 3' terminus, which said quencher is in close proximity to said fluorophore in the absence of cocaine; wherein cocaine binding to a first cocaine binding domain increases the affinity of the second cocaine binding domain; wherein the short and long fragment associate in the presence of cocaine thereby creating a rigid aptamer-cocaine structure that separates said quencher and said fluorophore; and wherein an increase in fluorescence is indicative of the presence of cocaine.

15. The cooperative-binding split aptamer, according to claim 14, wherein the quencher is an Iowa Black RQ black quencher and the fluorophore is Cy 5.

16. A method for detecting cocaine in a biological sample wherein said method comprises contacting said sample with a split aptamer of claim 12, and wherein a decrease in fluorescence of ATMND is indicative of the presence of cocaine.

17. The method, according to claim 16, wherein at least 76% of the fluorescence of ATMND fluorescence is quenched, after the sample is contacted with the aptamer.

18. The method, according to claim 16, wherein a signal gain of at least 6 is obtained in the presence of 25 μ M cocaine.

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